IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Lu-Kwang Ju

Serial No: 09/830,894

Filed: April 27, 2001

For: PRODUCTION OF BIOLOGICAL MATERIALS BY SIMULTANEOUS AEROBIC AND ANAEROBIC RESPIRATION

Group Art Unit: 1651

Confirmation No: 5277

Examiner: Irene Marx

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence was transmitted to the United States Patent and Trademark Office via facsimile on July 14, 2006 to phone number 571-273-8300.

Joseph J. Crimaldi

APPEAL BRIEF

Mail Stop Appeal Brief – Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

A single copy of this appeal brief is submitted in accordance with MPEP § 1205.02. No Extension of Time is believed due as a Notice of Appeal was filed on May 15, 2006. The Commissioner is hereby authorized to charge the small entity cost of filing this brief ($250) to Deposit Account No. 50-0959, Attorney Docket No. 089498.0338. Should any other fees be due, the Commissioner is hereby authorized to charge any such additional fees due to Deposit Account No. 50-0959.

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TABLE OF AUTHORITIES

Cases:

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I. Real Party in Interest:

The real party in interest in the present appeal is the Assignee, The University of Akron.

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II. Related Appeals and Interferences:

Appellant, Appellant's legal representatives, and/or the Assignee of the present patent application are unaware of any appeals and/or interferences that will directly affect, be directly affected by, or that will have a bearing on the Board's decision in the pending appeal.

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III. Status of Claims:

Claims 1 through 4, 6 through 34 and 106 through 108 are pending and stand twice or more rejected. A copy of the claims is attached as Appendix A. Claims 5, 35 through 69, 71 through 105, 109 and 110 were previously cancelled. Claims 70, 111 and 112 have been recently cancelled by an amendment filed on July 11, 2006.

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IV. Status of Amendments:

The Examiner issued an Office Action in response to a RCE on February 15, 2006. A Notice of Appeal was filed in response to the Office Action dated February 15, 2006 on May 15, 2006. An amendment seeking to cancel claims 70, 111 and 112, and amend claim 25, was filed on July 11, 2006.

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V. Summary of Invention:

The present invention generally relates to processes for producing a biological product via the use of a microorganism, and for increasing the concentration of a microorganism in a medium.

Claim 1: Independent claim 1 generally relates to enhancing production yields of a biological product.[1] More particularly, claim 1 relates to producing such a product under conditions that address high demands for oxygen by including an alternative oxidant source such as nitrates or the like.[2] Thus, when oxygen demand exceeds supply, the microorganisms can, in addition to the primary oxygen supply, additionally use an alternative oxidant for cellular respiration.[3] As a result, larger cellular concentrations are possible, which in turn results in larger product yields.[4]

More specifically, claim 1 relates to a process for the production of a biological product by a microorganism. The process includes providing a microorganism that produces the biological product.[5] The provided microorganism uses oxygen and an alternative oxidant source for cellular respiration[6]. The process also includes providing a culture medium suitable for the growth of the microorganism, wherein the medium comprises at least one carbon source.[7] The process further includes inoculating the culture medium with a desired cellular concentration of the microorganism.[8] The process still further includes aerating the culture medium with oxygen at a maximum rate of oxygen replenishment.[9] Yet further, the process includes supplying the culture medium with a suitable amount of the alternative oxidant source.[10] According to the process of the present invention, the microorganism uses the alternative oxidant source to supplement the

1. Specification, p. 21, ln. 17 to 19.
2. Specification, p. 21, ln. 26 to p. 22, ln. 2.
3. Specification, p. 10, ln. 23 to p. 11 ln. 8; and p. 13, lns. 15 to 17.
4. Specification, p. 21, lns. 17 to 19.
5. Specification, p. 11, lns. 8 to 9.
6. Id.
7. Id.
8. Specification, p. 11, lns. 10 to 13.
9. Specification, p. 13, ln. 14.
10. Specification, p. 13, lns. 15 to 17.

available oxygen.[11] Thus, when oxygen is plentiful, the microorganism will use the available oxygen, and as oxygen becomes deficient it will begin and increasingly utilize an alternative oxidant.[12] The process further includes sustaining cells in the culture medium such that at least a portion of the population consumes the alternative oxidant during at least a portion of the production process.[13] The process still further includes maintaining the culture medium at a suitable pH and temperature for the microorganism.[14] Yet further, the process includes allowing the culture medium to incubate, thereby yielding a biological product.[15] Finally, the process includes recovering the biological product. Biological products within the scope of claim 1 are biosurfactants, biopolymers, and enzymes, while claim 108 is specifically directed to the production of at least one rhamnolipid.

11. Id.
12. Id.
13. Specification, p. 13, ln. 14.
14. Specification, p. 15, ln. 16 to p. 16, ln. 24.
15. Specification, p. 15, ln. 17 to 18.

VI. Grounds of Rejection to be Reviewed on Appeal:

The grounds of rejection on Appeal are as follows:

(a) The Examiner has rejected, at least twice, claims 1 through 4, 10, 16 through 19, 21, 28, 31 through 34, 106 and 107 under 35 U.S.C. § 102(b) as anticipated by Robertson et al.[16] in light of Brock[17]; and

(b) The Examiner has rejected, at least twice, claims 1 through 4, 6 through 34 and 106 through 108 under 35 U.S.C. § 103(a) as obvious over Robertson et al. in view of Wendt et al.,[18] Brock, and Wagner et al.[19]

It should be noted that the actual rejection listed in the February 15, 2006 Office Action rejected claims 1 through 4, 6 through 34, 70 and **105 through 112**. However, claims 105, 109 and 110 were cancelled prior to the issuance of the February 15, 2006 Office Action, while claims 70, 111 and 112 have been recently cancelled. Accordingly, the 35 U.S.C. § 103(a) rejection listed above is correct in view of the claims pending for purposes of this Appeal.

16. Robertson, Lesley A., et al.; Simultaneous Nitrification and Denitrification in Aerobic Chemostat Cultures of *Thiosphaera pantotropha*; Applied and Environmental Microbiology; Vol. 54, No. 11, pp. 2812 to 2818; (1988).
17. Brock, Thomas D.; Biology of Microorganisms (Third Edition); Prentice Hall, Inc.; pp. 20 to 31 (1979).
18. United States Patent No. 3,939,068.
19. United States Patent No. 4,814,272.

VII. Grouping of Claims for Purposes of Appeal:

For the purposes of this appeal the pending claims, claims 1 through 4, 6 through 34 and 106 through 108, are grouped as follows:

Group A: Claims 1 through 4, 6 through 34, and 106 through 108 stand or fall together.

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VIII. Arguments:

(a) Claims 1 through 4, 10, 16 through 19, 21, 28, 31 through 34, 106 and 107 are not anticipated (35 U.S.C. § 102(b)) by Robertson et al. in light of Brock.

Group A: The rejection of claims 1 through 4, 10, 16 through 19, 21, 28, 31 through 34, 106 and 107 under 35 U.S.C. § 102(b) as anticipated by Robertson et al.[20] in light of Brock[21] is improper and should be reversed for at least the following reasons.

The Examiner contends that Robertson et al. discloses a process of making a biological product with *T. pantotropha* that can utilize an alternative oxidant source when the amount of available oxygen is insufficient, and where the biological product made is a biopolymer such as a protein or an enzyme or a biosurfactant such as a fatty acid and/or lipid.

It is not entirely clear from the Office Action dated February 15, 2006 as to why Brock is applied as part of the pending 35 U.S.C. § 102(b) rejection. It should be noted that the section of Brock being applied relates to specific alternative oxidant sources. Since, the claims rejected under 35 U.S.C. § 102(b) do not specify the exact nature of the claimed alternative oxidant., the addition of Brock to the 35 U.S.C. § 102(b) rejection of claims 1 through 4, 10, 16 through 19, 21, 28, 31 through 34, 106 and 107 adds nothing and should be withdrawn.

Given the above, the pending 35 U.S.C. § 102(b) rejection will be treated as only based on the disclosure contained in Robertson et al.

Robertson et al. is directed to understanding metabolic processes by analyzing the products of such processes. More particularly, Robertson et al. discloses quantifying/measuring intracellular protein production and relating this data to various

20. Robertson, Lesley A., et al.; Simultaneous Nitrification and Denitrification in Aerobic Chemostat Cultures of *Thiosphaera pantotropha*; Applied and Environmental Microbiology; Vol. 54, No. 11, pp. 2812 to 2818; (1988).
21. Brock, Thomas D.; Biology of Microorganisms (Third Edition); Prentice Hall, Inc.; pp. 20 to 31 (1979).

growth conditions for the sole purpose of monitoring cell growth (increase in biomass). This fact is supported by the Materials and Methods section of Robertson et al. under the sub-section title "Biomass Analysis" (see page 2813 of Robertson et al.). As would be appreciated by those of ordinary skill in the art, it is common to quantify or measure intracellular protein production in order to determine how much and/or how fast cells can grow under a given set of conditions. Thus, just because Robertson et al. quantifies or measures intracellular protein production, it is not fair to assume that Robertson et al. discloses a process for producing a commercially relevant amount of at least one biological product from a microorganism.

It should be noted that the present patent application specifically uses the term "biological product" to mean products that result from the preparation and isolation of commercially relevant quantities of relatively pure biological compounds. In this context, Robertson et al. clearly fails to disclose any type of isolation and/or recovery of commercially relevant quantities of one or more biological products. The only technique disclosed in Robertson et al. is the use of the Biuret method to colorimetrically quantify non-specifically protein in a biomass. According to the Biuret method, biomass is sampled, appropriately diluted, a Biuret solution is added, and the protein in the sample is colorimetrically quantified. The concentration of protein in the biomass is back-calculated from the sample concentration. However, as would be apparent to one of ordinary skill in the art, the Biuret method is not considered a recovery method.

On the other hand, the present invention, as is recited in pending claim 1, relates to a process for producing a biological product from a microorganism including, among other steps, recovering a biological product produced from a microorganism.

In light of the above, it is clear that Robertson et al. fails to disclose, teach or suggest any type of recovery and/or isolation of a commercially useful biological product. More importantly, Robertson et al. fails to disclose, teach or suggest the production and recovery of at least one biological product (*e.g.*, a biosurfactant, biopolymer and/or enzyme) via a process that utilizes, in part, an alternative oxidant source for cellular respiration (emphasis added – see pending claim 1). Since, Robertson et al. fails to

disclose, teach or suggest the above-mentioned process of claim 1, Robertson et al. can not anticipate claims 1 through 4, 10, 16 through 19, 21, 28, 31 through 34, 106 and 107. Additionally, as is noted above, Brock adds nothing of value to the novelty rejection of claims 1 through 4, 10, 16 through 19, 21, 28, 31 through 34, 106 and 107. Accordingly, reversal of the above-noted novelty rejection is believed due and is respectfully requested.

(b) Claims 1 through 4, 6 through 34, 106 and 107 are not obvious (35 U.S.C. § 103(a)) over Robertson et al. in view of Wendt et al., Brock, and Wagner et al.

Group A: The rejection of claims 1 through 4, 6 through 34, 106 and 107 under 35 U.S.C. § 103(b) as obvious over Robertson et al. in view of Wendt et al.,[22] Brock, and Wagner et al.[23] is improper and should be reversed for at least the following reasons.

The Examiner contends that Robertson et al. discloses a process of making a biological product with *T. pantotropha* that can utilize an alternative oxidant source when the amount of available oxygen is insufficient, where the biological product made is a biopolymer such as a protein or an enzyme, or a biosurfactant such as a fatty acid and/or lipid. The Examiner then acknowledges that Robertson et al. has a number of deficiencies therein (*e.g.*, that Robertson et al. fails to disclose, teach or suggest using any of the bacteria from the genera listed in pending claim 6). However, the Examiner cites to Wendt et al., Brock, and Wagner et al. as making up for the deficiencies in Robertson et al.

As is noted above, Robertson et al. is directed to understanding metabolic processes by analyzing the products of such processes. More particularly, Robertson et al. discloses quantifying/measuring intracellular protein production and relating this data to various growth conditions for the sole purpose of monitoring cell growth (increase in biomass). This fact is supported by the Materials and Methods section of Robertson et al. under the sub-section title "Biomass Analysis" (see page 2813 of Robertson et al.). As

22. United States Patent No. 3,939,068.
23. United States Patent No. 4,814,272.

would be appreciated by those of ordinary skill in the art, it is common to quantify or measure intracellular protein production in order to determine how much and/or how fast cells can grow under a given set of conditions. Thus, just because Robertson et al. quantifies or measures intracellular protein production, it is not fair to assume that Robertson et al. discloses a process for producing a commercially relevant amount of at least one biological product from a microorganism.

Again, it is noted that the present patent application specifically uses the term "biological product" to mean products that result from the preparation and isolation of commercially relevant quantities of relatively pure biological compounds. In this context, Robertson et al. clearly fails to disclose any type of isolation and/or recovery of commercially relevant quantities of one or more biological products. The only technique disclosed in Robertson et al. is the use of the Biuret method to colorimetrically quantify non-specifically protein in a biomass. According to the Biuret method, biomass is sampled, appropriately diluted, a Biuret solution is added, and the protein in the sample is colorimetrically quantified. The concentration of protein in the biomass is back-calculated from the sample concentration. However, as would be apparent to one of ordinary skill in the art, the Biuret method is not considered a recovery method.

Wendt et al. relates to a process for treating waste water containing cellulose nitrate particles. The process disclosed in Wendt et al. involves the use of a microorganism to denitrify waste water. Wendt et al. is not concerned with, nor does it disclose, teach or suggest, any type of production and/or of a commercially useful biological product.

In response to the Examiner's argument that Wendt et al. produces a biological product, namely water, Applicant would like to point out that Wendt et al. is not producing water. Rather, Wendt et al. is only concerned with the removal of cellulose nitrate particles from a waste water stream. In other words, Wendt et al. is only concerned with purifying and/or "cleaning" a waste water stream, not with the production of one or more biological products from a microorganism. Accordingly, Wendt et al. fails to cure the deficiencies of Robertson et al.

Brock is a text book that, among other things, discusses anaerobic respiration in microorganisms. As such, Brock is not concerned with, nor does it disclose, teach or suggest, the production and recovery of at least one biosurfactant, biopolymer, protein, and/or enzyme (emphasis added – see pending claim 1). Accordingly, Brock fails to cure the deficiencies of Robertson et al.

Wagner et al. relates to the production anionic rhamnolipids via the use of a microorganism. However, as is disclosed in Wagner et al., the process disclosed therein is solely based on aerobic respiration. As such, Wagner et al. is not concerned with, nor does it disclose, teach or suggest, the production and recovery of at least one biological product (*e.g.*, a biosurfactant, biopolymer and/or enzyme) via a process that utilizes, in part, an alternative oxidant source for cellular respiration (emphasis added – see pending claim 1). Accordingly, Wagner et al. fails to cure the deficiencies of Robertson et al.

Thus, for at least the above reasons, it is clear that Robertson et al. in combination with any one or more of Wendt et al., Brock, and Wagner et al. fails to disclose, teach or suggest a method for producing and recovering of at least one biological product (*e.g.*, a biosurfactant, biopolymer and/or enzyme) via a process that utilizes, in part, an alternative oxidant source for cellular respiration (emphasis added – see pending claim 1). Given this, Robertson et al. in combination with any one or more of Wendt et al., Brock, and Wagner et al. can not render obvious claims 1 through 4, 6 through 34, and 106 through 108.

Furthermore, even were the Board of Appeals to concluded that the claimed elements of the present invention are disclosed individually in the art cited by the Examiner (a position Applicant/Appellant does not believe is supported by the record), no *prima facie* case of obviousness has been established as is necessary in view of the Federal Circuit's decision in In re Kotzab, 55 USPQ2d 1313 (Fed. Cir. 2000). This is because no evidence has been shown of a motivation, suggestion or teaching to combine the cited art in the manner suggested by the Examiner. As was stated by the Federal Circuit in In re Kotzab:

> Most if not all inventions arise from a combination of old elements. . . . Thus, every element of a claimed invention may often be found in the prior art. . . . However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. . . . Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant.

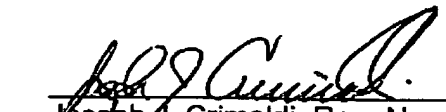
Id. at 1317. Given the clear lack of evidence of motivation to combine the cited art in the manner suggested by the Examiner, the present invention as recited in claims 1 through 4, 6 through 34, and 106 through 108 is patentable over the cited art.

Accordingly, for at least the above reasons, reversal of the above-noted obviousness rejection is believed due and is respectfully requested.

Conclusion:

For the foregoing reasons, Appellant respectfully submits that the claimed invention is not anticipated by the combination of Robertson et al. and Brock. Nor is the claimed invention rendered obvious by the combination of Robertson et al. with any one or more of Wendt et al., Brock, and Wagner et al. This honorable Board is requested to reverse the Examiner's rejections of all of the claims pending in the application and to allow these claims.

Respectfully submitted,



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IX. Appendix: Claims:

1. (previously presented) A process for the production of a biological product by a microorganism comprising the steps of:

providing a microorganism that produces the biological product, and uses oxygen and an alternative oxidant source other than oxygen for cellular respiration;

providing a culture medium suitable for the growth of the microorganism, wherein the medium comprises at least one carbon source;

inoculating the culture medium with a desired cellular concentration of the microorganism;

aerating the culture medium with oxygen, wherein the process has a maximum oxygen replenishment rate to the culture medium;

supplying the culture medium with a suitable amount of the alternative oxidant source that can be used by the microorganism to permit cellular respiration such that when the oxygen requirement for cellular respiration of the microorganism within the culture medium is less than the maximum rate of oxygen replenishment to the culture medium, the microorganisms will substantially utilize oxygen for cellular respiration, and when the oxygen requirements for cellular respiration of the microorganisms within the culture medium is greater than the maximum rate of oxygen supply to the culture medium, then at least a portion of the microorganism concentration within the culture medium will utilize the alternative oxidant source for cellular respiration;

sustaining cells in the culture medium such that at least a portion of the population consumes the alternative oxidant during at least a portion of the production process;

maintaining the culture medium at a suitable pH and temperature for the microorganism;

allowing the culture medium to incubate, thereby yielding a biological product; and

recovering the biological product,

wherein the biological product is at least one biological product selected from biosurfactants, biopolymers, and enzymes.

2. (previously presented) The process of claim 1, wherein the step of recovering the biological product further comprises the step of isolating the biological product from the culture medium.

3. (original) The process of claim 1, wherein the microorganism is selected from the group consisting of bacteria, yeasts, molds and archaea.

4. (original) The process of claim 3, wherein the microorganism is a bacteria.

5. (canceled)

6. (previously presented) The process of claim 4, wherein the bacteria is selected from a genus selected from the group consisting of *Pseudomonas, Paracoccus, Micrococcus, Klebsiella, Escherichia, Acidianus, Campylobactor, Wolinella,* and *Proteus.*

7. (original) The process of claim 6, wherein the genus is *Pseudomonas.*

8. (original) The process of claim 7, wherein the species of the genus *Pseudomonas* is selected from the group consisting of *Pseudomonas aeruginosa, Pseudomonas fluorescens, Pseudomonas putida, Pseudomonas cruciviae, Pseudomonas boreopolis and Pseudomonas oleovorans.*

9. (original) The process of claim 8, wherein the species of *Pseudomonas* is *Pseudomonas aeruginosa.*

10. (previously presented) The process of claim 1, wherein the at least one carbon source is selected from the group consisting of fatty acids, glycerol, low molecular weight acids, carbohydrates, yeast extract, peptone and vegetable oil.

11. (original) The process of claim 10, wherein the fatty acids are selected from the group consisting of palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, butyric acid, caproic acid, lauric acid, and linolenic acid.

12. (original) The process of claim 11, wherein the fatty acid is palmitic acid.

13. (original) The process of claim 10, wherein the vegetable oil is selected from the group consisting of corn oil, peanut oil, coconut oil, linseed oil, olive oil, soy bean oil and sunflower oil.

14. (original) The process of claim 13, wherein the vegetable oil is corn oil.

15. (original) The process of claim 10, wherein the carbohydrate is glucose.

16. (original) The process of claim 10, wherein the low molecular weight acid is selected from the group consisting of malate, acetate and pyruvate.

17. (original) The process of claim 1, wherein the alternative oxidant source is selected from the group consisting of nitrates, nitrites, sulfates, sulfites, carbonates, fumarates, sulfur, manganic ion, ferric ion, selenate, dimethyl sulfoxide, arsenate, trimethylamine N-oxide and glycine.

18. (original) The process of claim 17, wherein the alternative oxidant source is a nitrate.

19. (original) The process of claim 18, wherein the nitrate is selected from the group consisting of sodium nitrate, potassium nitrate, calcium nitrate, magnesium nitrate, ammonium nitrate, and nitric acid.

20. (original) The process of claim 19, wherein the nitrate is sodium nitrate.

21. (original) The process of claim 17, wherein the nitrites are selected from the group consisting of sodium nitrite, potassium nitrite, calcium nitrite, ammonium nitrite, and nitrous acid.

22. (original) The process of claim 17, wherein the sulfates are selected from the group consisting of sodium sulfate, potassium sulfate, calcium sulfate, iron sulfate, magnesium sulfate, ammonium sulfate, zinc sulfate, copper sulfate, cobalt sulfate, manganese sulfate, and dilute sulfuric acid.

23. (original) The process of claim 17, wherein the sulfites are selected from the group consisting of calcium sulfite, sodium sulfite, potassium sulfite, iron sulfite, magnesium sulfite, ammonium sulfite, zinc sulfite, copper sulfite, cobalt sulfite and manganese sulfite.

24. (original) The process of claim 17, wherein the carbonates are selected from the group consisting of calcium carbonate, sodium carbonate, and potassium carbonate.

25. (original) The process of claim 17, wherein the bicarbonates are selected from the group is consisting of calcium bicarbonate, sodium bicarbonate, and potassium bicarbonate.

26. (original) The process of claim 17, wherein the fumarates are selected from the group consisting of disodium fumarate, sodium fumarate, dipotassium fumarate, potassium fumarate, and fumaric acid.

27. (previously presented) The process of claim 1, further comprising the step of adding a sufficient amount of a surfactant to the culture medium to facilitate the mass transfer of the carbon source into the culture medium.

28. (original) The process of claim 1, further comprising the step of limiting an essential growth nutrient from the culture medium.

29. (original) The process of claim 28, wherein the essential growth nutrient is selected from the group consisting of phosphorous, nitrogen, sulfur, calcium, magnesium and iron.

30. (original) The process of claim 29, wherein the essential growth nutrient is phosphorous.

31. (previously presented) The process of claim 1, wherein the cellular concentration of the microorganism is from about 0.1 g/L to about 50 g/L.

32. (original) The process of claim 1, wherein the concentration of the alternative oxidant source in the culture medium is in the range of from about 0.01 to about 10 g/L.

33. (original) The process of claim 1, wherein the culture is maintained in a temperature range of about 20°C to about 40°C.

34. (original) The process of claim 1, wherein the culture is maintained in a pH range of about 4 to about 9.

Claims 35 through 105 (cancelled).

106. (previously presented) The process of claim 1, wherein the biological product is at least one biosurfactant.

107. (previously presented) The process of claim 106, wherein the at least one biosurfactant is selected from one or more rhamnolipids, sophorolipids, trehalose mycolates, trehalose esters, monosaccharide mycolates, disaccharide mycolates, trisaccharide mycolates, phospholipids, fatty acids, ornithinelipids, lysine-lipids, surfactins, peptide-lipids, heteropolysaccharides, manno-proteins, carbohydrate-proteins, mannan-lipid complexes, mannose/erythrose-lipids, and carbohydrate-protein-lipid complexes.

108. The process of claim 1, wherein the biological product is at least one rhamnolipid.

Claims 109 through 112 (cancelled).

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X. Appendix: Evidence:

None.

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XI. Appendix: Related Proceedings:

None.

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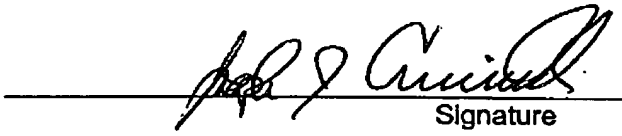

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